AMENDMENTS TO THE SPECIFICATION

Please amend the last paragraph on page 1 through page 2 as follows:

Similarly, electronic detection of nucleic acids using electrodes is also known; see for example U.S. Pat. Nos. 5,591,578; 5,824,473; 5,705,348; 5,780,234, ~~and~~ 5,770,369, 5,952,172 and 6,232,062; ~~U.S. Ser. Nos. 08/873,598 08/911,589;~~ and WO 98/20162; ~~PCT/US98/12430~~ WO 98/57159; ~~PCT/US98/12082~~ WO 98/57158; ~~PCT/US99/10104~~ WO 99/57317; ~~PCT/US99/01705~~ WO 99/37819, and ~~PCT/US99/01703~~ WO 99/57319.

Please amend the second paragraph on page 5 as follows:

In general, any assay methods that rely on electrochemical detection may benefit from the techniques of the present invention. For example, the methods of the invention find use in systems that do not utilize monolayers, as well as those that do not use exogeneous ETMs and in systems that rely on techniques other than AC. However, the present invention finds particular use in systems such as are generally described in U.S. Pat. Nos. 5,591,578; 5,824,473; 5,705,348; 5,780,234, ~~and~~ 5,770,369, 5,952,172 and 6,232,062; ~~U.S. Ser. Nos. 08/873,598 08/911,589;~~ and WO 98/20162; ~~PCT/US98/12430~~ WO 98/57159; ~~PCT/US98/12082~~ WO 98/57158; ~~PCT/US99/10104~~ WO 99/57317; ~~PCT/US99/01705~~ WO 99/37819, and ~~PCT/US99/01703~~, WO 99/57319. These systems rely on the use of capture binding ligands (called capture probes when the target analyte is a nucleic acid) to anchor target analytes to the electrode surface and form an assay complex. The assay complex further comprises an electron transfer moiety (ETM), that is directly or indirectly attached to the target analyte. That is, the presence of the ETM near the electrode surface is dependent on the presence of the target analyte. Electron transfer between the ETM and the electrode is initiated using a variety of techniques as outlined below, and the output signals received and optionally processed as further outlined below. Thus, by detecting electron transfer, the presence or absence of the target analyte is determined.

Please amend the last paragraph on 6 through page 7 as follows:

Accordingly, the present invention provides methods of detecting a target analyte in sample solutions. As will be appreciated by those in the art, the sample solution may comprise any number of things, including, but not limited to, bodily fluids (including, but not limited to, blood, urine, serum, lymph, saliva, anal and vaginal secretions, perspiration and semen, of virtually any organism, with mammalian samples being preferred and human samples being particularly preferred); environmental samples (including, but not limited to, air, agricultural, water and soil samples); biological warfare agent samples; research samples (i.e. in the case of nucleic acids, the sample may be the products of an amplification reaction, including both target and signal amplification as is generally described in ~~PCT/US99/01705~~ WO 99/37819, such as PCR amplification reaction); purified samples, such as purified genomic DNA, RNA, proteins, etc.; raw samples (bacteria, virus, genomic DNA, etc.; As will be appreciated by those in the art, virtually any experimental manipulation may have been done on the sample.

Please amend the second full paragraph on page 33 as follows:

In this embodiment, when the binding ligand is a nucleic acid, preferred compositions and techniques are outlined in WO 98/20162; ~~PCT/US98/12430~~ WO 98/57159; ~~PCT/US98/12082~~ WO 98/57158; ~~PCT/US99/01705~~ WO 99/37819; ~~PCT/US99/01703~~ WO 99/57319; and U.S. Ser. ~~Nos~~ No. ~~09/135,183;~~ 60/105,875; and ~~09/295,691~~ U.S. Patent Nos. 7,090,804 and 6,942,771, all of which are hereby expressly incorporated by reference.

Please amend the last paragraph on page 71 through page 72 as follows:

The assays are generally run under stringency conditions which allows formation of the label probe hybridization complex only in the presence of target. Stringency can be controlled by altering a step parameter that is a thermodynamic variable, including, but not limited to,

temperature, formamide concentration, salt concentration, chaotropic salt concentration pH, organic solvent concentration, etc. Stringency may also include the use of an electrophoretic step to drive non-specific (i.e. low ~~stringency~~ stringency) materials away from the detection electrode, just as electrophoresis can be used to bind the target analytes to their binding ligands, as is described in ~~U.S. Ser. No. 09/134,058~~ U.S. Patent No. 6,290,839, hereby expressly incorporated by reference.

Please amend paragraph five on page 73 as follows:

The attachment of the conductive oligomer to the nucleoside may be done in several ways. In a preferred embodiment, all or part of the conductive oligomer is synthesized first (generally with a functional group on the end for attachment to the electrode), which is then attached to the nucleoside. Additional nucleosides are then added as required, with the last step generally being attachment to the electrode. Alternatively, oligomer units are added one at a time to the nucleoside, with addition of additional nucleosides and attachment to the electrode. A number of representative syntheses are shown in the Figures of ~~PCT US97/20014~~ WO 98/20162, expressly incorporated herein by reference.

Please amend the paragraphs 1-3 on page 74 as follows:

In a preferred embodiment, attachment is to a ribose of the ribose-phosphate backbone. Thus, attachment via amide and amine linkages are possible (see FIGS. 1 and 2 of ~~CPT US97/20014~~ WO 98/20162). In a preferred embodiment, there is at least a methylene group or other short aliphatic alkyl groups (as a Z group) between the nitrogen attached to the ribose and the aromatic ring of the conductive oligomer. A representative synthesis is shown in FIG. 16 of ~~PCT US97/20014~~ WO 98/20162.

Alternatively, attachment is via a phosphate of the ribose-phosphate backbone. Examples of two synthetic schemes are shown in FIG. 4 and FIG. 5 of ~~PCT US97/20014~~ WO 98/20162. Although both Figures show attachment at the 3' position of the ribose, attachment can also be made via the 2' position. In FIG. 5, Z is an ethylene linker, although other linkers may be used as well, as will be appreciated by those in the art.

In a preferred embodiment, attachment is via the base. A general scheme is depicted in FIG. 3 of ~~PCT US97/20014~~ WO 98/20162, using uridine as the nucleoside and a phenylene-acetylene conductive oligomer. As will be appreciated in the art, amide linkages are also possible, using techniques well known in the art. In a preferred embodiment, protecting groups may be added to the base prior to addition of the conductive oligomers, as is generally outlined in FIGS. 10 and 11 of ~~PCT US97/20014~~ WO 98/20162. In addition, the palladium cross-coupling reactions may be altered to prevent dimerization problems; i.e. two conductive oligomers dimerizing, rather than coupling to the base.

Please amend paragraph 4 on page 76 as the follows:

In a preferred embodiment, ETMs are attached to a phosphate of the ribose-phosphate backbone. As outlined herein, this may be done using phosphodiester analogs such as phosphoramidite bonds, see generally PCT publication WO 95/15971, or can be done in a similar manner to that depicted in FIGS. 4 and 5 of ~~PCT US97/20014~~ WO 98/20162, where the conductive oligomer is replaced by a transition metal ligand or complex or an organic ETM, as well as is outlined in the Examples.

Please amend the first full paragraph on page 79 as the follows:

In a preferred embodiment, the nucleic acid is a peptide nucleic acid or analog. In this embodiment, the invention provides peptide nucleic acids with at least one covalently attached

ETM or attachment linker. In a preferred embodiment, these moieties are covalently attached to ~~an~~ a monomeric subunit of the PNA. By "monomeric subunit of PNA" herein is meant the -NH-CH_2CH_2-N($COCH_2$-Base)-CH_2-CO-monomer, or derivatives (herein included within the definition of "nucleoside") of PNA. For example, the number of carbon atoms in the PNA backbone may be altered; see generally Nielsen et al., Chem. Soc. Rev. 1997 page 73, which discloses a number of PNA derivatives, herein expressly incorporated by reference. Similarly, the amide bond linking the base to the backbone may be altered; phosphoramide and sulfuramide bonds may be used. Alternatively, the moieties are attached to an internal monomeric subunit. By "internal" herein is meant that the monomeric subunit is not either the N-terminal monomeric subunit or the C-terminal monomeric subunit. In this embodiment, the moieties can be attached either to a base or to the backbone of the monomeric subunit. Attachment to the base is done as outlined herein or known in the literature. In general, the moieties are added to a base which is then incorporated into a PNA as outlined herein. The base may be either protected, as required for incorporation into the PNA synthetic reaction, or derivatized, to allow incorporation, either prior to the addition of the chemical substituent or afterwards. Protection and derivatization of the bases is shown in FIGS. 24-27 of ~~PCT US97/20014~~ WO 98/20162. The bases can then be incorporated into monomeric subunits as shown in FIG. 28 of ~~PCT US97/20014~~ WO 98/20162. FIGS. 29 and 30 of ~~PCT US97/20014~~ WO 98/20162 depict two different chemical substituents, an ETM and a conductive oligomer, attached at a base. FIG. 29 depicts a representative synthesis of a PNA monomeric subunit with a ferrocene attached to a uracil base. FIG. 30 depicts the synthesis of a three unit conductive oligomer attached to a uracil base.

Please amend the first full paragraph on page 80 as follows:

In this embodiment, a modified monomeric subunit is synthesized with an ETM or an attachment linker, or a functional group for its attachment, and then the base is added and the modified monomer can be incorporated into a growing PNA chain. FIG. 31 of ~~PCT US97/20014~~ WO 98/20162 depicts the synthesis of a conductive oligomer covalently attached to the backbone of a

PNA monomeric subunit, and FIG. 32 of ~~PCT US97/20014~~ WO 98/20162 depicts the synthesis of a ferrocene attached to the backbone of a monomeric subunit.

Please amend page 122 though line 9 of page 123 as the follows:

As above, the circuit boards were removed from the foil-lined bags and immersed in a 10% sulfuric acid solution for 30 seconds. Following the sulfuric acid treatment, the boards were immersed in two Milli-Q water baths for 1 minute each. The boards were then dried under a stream of nitrogen. The boards were placed on a X-Y table in a humidity chamber and a 30 nanoliter drop of DNA deposition solution was placed on each of the 14 electrodes. The DNA deposition solution consisted of 33 ~~uM~~ μM thiolated DNA, 33 ~~uM~~ μM 2-unit phenylacetylene wire (H6), and 16 ~~uM~~ μM undec-1-en-11yltri(ethylene glycol)$(HS\text{-}CH_2)_{11}$-$(OCH_2CH_2)_3$-OH) in 6x SSC (900 mM sodium chloride, 90 mM sodium Citrate, pH 7) w/1% Triethylamine. 3 electrodes were spotted with a solution containing DNA 1 (SEQ ID NO: 1) (5'-ACCATGGACACAGAT$(CH_2)_{16}$SH-3'). 4 electrodes were spotted with a solution containing DNA 2 (SEQ ID NO: 2) (5'TCATTGATGGTCTCTTTTAACA$(CH_2)_{16}$SH-3'). 4 electrodes were spotted with DNA 3 (SEQ ID NO: 3) (5'CACAGTGGGGGGACATCAAGCAGCCATGCAAA$(CH_2)_{16}$SH-3'). 3 electrodes were spotted with DNA 4 (SEQ ID NO: 4) (5'-TGTGCAGTTGACGTGGAT$(CH_2)_{16}$SH-3'). The deposition solution was allowed to incubate at room temperature for 5 minutes and then the drop was removed by rinsing in a Milli-Q water bath. The boards were immersed in a 45 °C bath of M44 in acetonitrile. After 30 minutes, the boards were removed and immersed in an acetonitrile bath for 30 seconds followed by a milli-Q water bath for 30 seconds. The boards were dried under a stream of nitrogen and stored in foiled-lined bags flushed with nitrogen until use.

The modified boards were removed from the foil-lined bags and fitted with an injection molded sample chamber (cartridge). The chamber was adhered to the board using double-sided sticky tape and had a total volume of 250 microliters. A hybridization solution was prepared. The

solution contains 10 nM DNA target (SEQ ID NO: 5) (5'-TGTGCAGTTGACGTGGATTGTTAAAAGAGACCATC[[M]]AATGAGG[[M]]AAGCTGCAG[[M]]AATGGGATAGAGTCATCCAGT-3') (D-998), 30 nM signaling probe (D-1055) (SEQ ID NO: 7) and 10 nm (SEQ ID NO: 6) (5'-TCTACAG(N6)C(N6)ATCTGTGTCCATGGT-3') (N6 is shown in FIG. 1D of ~~PCTUS99/01705~~ WO 99/37819; it comprises a ferrocene connected by a 4 carbon chain to the 2' oxygen of the ribose of a nucleoside). The ~~signalling~~ signaling probe is as follows:

5'-$(C23)_4$-N87-N87-N87-N87-ATC CAC GTC AAC TGC ACA-3' (D-1055) (SEQ ID NO: 7)

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C23 C23 C23 C23

C23 C23 C23 C23

C23 C23 C23 C23

C23 C23 C23 C23

N87 is a branch point comprising a ring structure. C23 is shown in FIG. 1F of ~~PCTUS99/01705~~ WO 99/37819. In a solution containing 25% Qiagen lysis buffer AL, 455 mM $NaClO_4$, 195 mM NaCl, 1.0 mM mercaptohexanol and 10% fetal calf serum. 250 microliters of hybrid solution was injected into the cartridge and allowed to hybridize for 12 hours. After 12 hours, the hybridized chip was plugged into a homemade transconductance amplifier with switching circuitry. The transconductance amplifier was equipped with summing circuitry that combines a DC ramp from the computer DAQ card and an AC sine wave from the lock-in amplifier (SR830 Stanford Instruments). Each electrode was scanned sequentially and the data was saved and manipulated using a homemade program designed using Labview (National Instrents). The chip was scanned at between -100 mV and 500 mV (pseudo Ag/Ag/Cl reference electrode) DC with a 25 mV (50 mV peak to peak), 1000 Hz superimposed sine wave. The output current was fed into the lock-in amplifier and the 1000 Hz signal was recorded (ACV technique). The data for each set of pads was compiled and averaged.

Please add the Sequence Listing on page 128, immediately preceding the claims.